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L5: Entry 1 of 4 File: USPT Dec 15, 1998

DOCUMENT-IDENTIFIER: US 5849690 A
TITLE: Anti-aggregatory peptides

BSPR:
Recent advances for treatment of occluded arteries and deep vein thrombosis employ fibrinolytic agents to lyse thrombi or emboli in order to reestablish or improve blood flow. Fibrinolytic agents are proteolytic enzymes which hydrolyze fibrin at specific sites and thereby fragment the fibrin network. Fragmentation of fibrin into smaller peptides has the effect of solubilizing the thrombus or embolus. Tissue plasminogen activator (tPA), urokinase (UK) and pro-Urokinase(pUK), which are of human origin, and streptokinase (SK), which is of bacterial origin, are considered fibrinolytic agents within the context of this disclosure. Their action in vivo is to proteolytically activate plasminogen in the blood to form plasmin, which is the actual fibrinolytic agent. Of these, tPA and SK are commercially used for fibrinolytic therapy. A recurrent problem with such therapy, however, is the reocclusion of the blood vessel due to formation of a secondary thrombus.

DRPR:
FIG. 2 demonstrates the inhibition of platelet aggregation in vivo by infusion of 400 mM Ac-RGDS-NH.sub.2 at a rate of 0.1 ml/min. Platelet aggregation/thrombus formation is indicated by a reduction in blood flow. Arrows indicate initiation and termination of infusion. SL (shake loose) indicates mechanical dislodgement of the thrombus. Top trace (a) displays the coronary arterial blood pressure (mmHg) as a function of time, which indicates no effect of infusion of Ac-RGDS-NH.sub.2 upon blood pressure. Middle trace (b) demonstrates the variation of phasic coronary blood flow (ml/min) as a function of time, which indicates inhibition of thrombus formation after infusion of the peptide. Lower trace (c) displays the mean coronary blood flow (ml/min) as a function of time, which indicates inhibition of thrombus formation after infusion of the peptide.

DRPR:
FIGS. 3A-3C demonstrate the dose dependency of in vivo inhibition of platelet aggregation in the coronary thrombosis model. The graph displays mean coronary blood flow as a function of time. Arrows indicate the initiation and termination of infusion of Ac-RGDS-NH.sub.2. Thrombus formation is indicated by a decrease in blood flow. SL (shake loose) indicates a mechanical dislodgement of a thrombus, X indicates spontaneous sloughing off of a thrombus. The top trace (a) indicates complete inhibition of

thrombus formation (400 mM at 0.052 ml/min. infusion rate). The middle trace (b) indicates moderate inhibition with spontaneous sloughing off of thrombi (400 mM at 0.026 ml/min. infusion). The bottom trace (c) shows partial inhibition with prolongation of time to complete blockage, requiring mechanical dislodgement of thrombus (400 mM at 0.013 ml/min.).

DEPR:
This invention also provides a method of inhibiting platelet aggregation and clot formation in a mammal, especially a human, in need thereof, which comprises the internal administration of an effective amount of the antifibrotic peptide and a pharmaceutically acceptable carrier. Indications for such therapy include myocardial infarction, deep vein thrombosis, pulmonary embolism, dissecting anurysm, stroke and other infarct-related disorders. Chronic or acute states of hyper-aggregability, such as disseminated intravascular coagulation (DIC), septicemia, surgical or infectious shock, post-operative and post-partum trauma, cardiopulmonary bypass surgery, incompatible blood transfusion, abruptio placenta, thrombotic thrombocytopenic purpura (TTP), snake venom and immune diseases, are likely to be responsive to such treatment. The anti-fibrotic peptide is administered either orally or parenterally to the patient, in a manner such that the concentration of drug in the plasma is sufficient to inhibit platelet aggregation. The pharmaceutical composition containing the peptide is administered at a dose between about 0.2 to about 50 mg/kg in a manner consistent with the condition of the patient. For acute therapy, parenteral administration is preferred. For persistant states of hyperaggregability, an intravenous infusion of the peptide in 5% dextrose in water or normal saline is most effective, although an intramuscular bolus injection may be sufficient.

DEPR:
Indications for such therapy include myocardial infarction, deep vein thrombosis, pulmonary embolism, stroke and other infarct-related disorders. The anti-fibrotic is administered just prior to, at the same time as, or just after parenteral administraton of tPA or other fibrinolytic agent. It may prove desirable to continue treatment with the anti-fibrotic for a period of time well after reperfusion has been established to maximally inhibit post-therapy reocclusion. The effective dose of tPA, SK, UK or pUK may be from 0.5 to 5 mg/kg and the effective dose of the anti-fibrotic peptide may be from about 0.1 to 25 mg/kg.

DEPR:
In vivo inhibition of thrombus formation is demonstrated by recording the systemic and hemodynamic effects of infusion of the peptides into anesthetized dogs according to the methods described in Aiken et al., Prostaglandins, 19, 629-43 (1980). Briefly, a small Lexan.RTM. cylinder is placed around a mechanically damaged left circumflex coronary artery to produce a fixed partial obstruction of 80-90%. Under these conditions, platelets adhere to the exposed subendothelial collagen and aggregate at the obstructed site. Aggregation is assessed as a gradual reduction in blood flow over 2-3 min. until the thrombus

is mechanically dislodged from the lumen of the obstructed vessel and coronary blood flow is restored. This process is allowed to repeat itself every 2-3 min. through the control period of the experiment.

DEPR:
The results of such an experiment using Ac-RGDS-NH.sub.2 are illustrated in FIG. 2 (a-c). Thus the top trace, (a), measures arterial blood pressure (mmHg), the middle trace, (b), measures phasic coronary blood flow (ml/min.) and the bottom trace, (c), measures mean coronary blood flow. During the control period, flow decreases (trace (b) and (c)) until the clot is shaken loose (SL). The arrow indicates initiation of coronary infusion of Ac-RGDS-NH.sub.2 (400 mm at 0.1 ml/mm). This infusion resulted in complete inhibition of thrombus formation until infusion was terminated (second arrow). Termination of infusion results in a decrease in flow as thrombus formation occurs.

DEPR:
Dose dependence of the anti aggregatory activity is demonstrated by observing the effect upon mean coronary blood flow (ml/mm) when the infusion rate of the anti-fibrotic peptide is varied. This is demonstrated in FIG. 3 (a-c) for Ac-RGDS-NH.sub.2. The top trace, (a), shows complete inhibition of thrombus formation (400 mM at 0.052 ml/min.). The middle trace, (b), shows moderate inhibition with spontaneous sloughing off of the thrombus X (400 mM at 0.026 ml/min.). The bottom trace, (c), shows partial inhibition with prolongation of time to complete blockage (400 mM, 0.013 ml/min.), which required the thrombus to be shaken loose (SL). Again initiation and termination of infusion is depicted by arrows.

CLPR:
5. A method for preventing or treating myocardial infarction in a mammal comprising administering an effective amount of a peptide according to claim 1, and a pharmaceutically acceptable carrier.

L5: Entry 3 of 4 File: USPT Nov 28, 1995

DOCUMENT-IDENTIFIER: US 5470849 A
TITLE: .gamma.-turn peptidomimetics as fibrinogen antagonists

BSPR:
Recent advances for treatment of occluded arteries and deep vein thrombosis employ fibrinolytic agents to lyse thrombi or emboli in order to reestablish or improve blood flow. Fibrinolytic agents, such as anistreplase, tissue plasminogen activator (tPA), urokinase (UK), pro-Urokinase(pUK), and streptokinase (SK), and mutants and derivatives thereof, are proteolytic enzymes which cause fibrin to be hydrolyzed at specific sites and thereby fragment the fibrin network. Lysis of fibrin into smaller peptides has the effect of solubilizing the thrombus or embolus. A recurrent problem with such therapy, however, is the reocclusion of the blood vessel due to formation of a secondary thrombus.

DEPR:
This invention also provides a method of inhibiting platelet aggregation and clot formation in a mammal, especially a human, in need thereof, which comprises the internal administration of a compound according to formula (II) and a pharmaceutically acceptable carrier. Indications for such therapy include myocardial infarction, deep vein thrombosis, pulmonary embolism, dissecting anurysm, transient ischemia attack (TIA), stroke and other infarct-related disorders. Chronic or acute states of hyper-aggregability, such as disseminated intravascular coagulation (DIC), septicemia, surgical or infectious shock, post-operative and post-partum trauma, cardiopulmonary bypass surgery, incompatible blood transfusion, abruptio placenta, thrombotic thrombocytopenic purpura (TTP), snake venom and immune diseases, are likely to be responsive to such treatment. In addition, the peptides of this invention may be used in a method for the prevention of metastatic conditions.

DEPR:
Indications for such therapy include myocardial infarction, deep vein thrombosis, pulmonary embolism, stroke and other infarct-related disorders. The compound of this invention is administered just prior to, at the same time as, or just after parenteral administration of tPA or other fibrinolytic agent. It may prove desirable to continue treatment with the compounds of this invention for a period of time well after reperfusion has been established to maximally inhibit post-therapy reocclusion. The effective dose of tPA, SK, UK or pUK may be from 0.1 to 5 mg/kg and the effective dose of the compounds of this invention may be from about 0.1 to 25 mg/kg.

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L9: Entry 3 of 5 File: USPT Jul 6, 1999

DOCUMENT-IDENTIFIER: US 5919452 A
TITLE: Methods of treating TNF.alpha.-mediated disease using chimeric anti-TNF antibodies

DRPR:
FIG. 15 is an amino acid sequence of human TNF showing sequences having portions of epitopes recognized by cA2, corresponding to portions of amino acids 59-80 and/or 87-108 of SEQ ID NO:1.

DEPR:
Preferred anti-TNF mAbs are also those which will competitively inhibit in vivo the binding to human TNF.alpha. of anti-TNF.alpha. murine mAb A2, chimeric mAb cA2, or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Preferred antibodies of the present invention are those that bind epitopes recognized by A2 and cA2, which are included in amino acids 59-80 and/or 87-108 of hTNF.alpha. (as these corresponding amino acids of SEQ ID NO:1), such that the epitopes consist of at least 5 amino acids, which comprise at least one amino acid from the above portions of human TNF.alpha..

DEPR:
In an alternative way of cloning a polynucleotide encoding an anti-TNF variable or constant region, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing an anti-TNF antibody or variable or constant region) into an expression vector. The library is then screened for members capable of expressing a protein which competitively inhibits the binding of an anti-TNF antibody, such as A2 or cA2, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as anti-TNF antibodies or fragments thereof. In this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing an anti-TNF antibody or fragment. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment such as in a lambda phage library, expression in prokaryotic cell (e.g., bacteria) or eukaryotic cells, (e.g., mammalian, yeast, insect or fungus). See, e.g., Ausubel, infra, Harlow, infra, Colligan, infra; Nyyssonen et al. Bio/Technology 11:591-595 (Can 1993); Marks et al., Bio/Technology 11:1145-1149 (October 1993). Once nucleic acid

may be from about 0.1 to 25 mg/kg.